



Taxonomic Paper

First national record of *Gracixalus quangii* Rowley, Dau, Nguyen, Cao & Nguyen, 2011 and *G. yunnanensis* Yu, Li, Wang, Rao, Wu & Yang, 2019 (Amphibia: Anura: Rhacophoridae) from Thailand

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Abstract

Background

The bushfrog genus *Gracixalus* Delorme, Dubois, Grosjean & Ohler, 2005 is found in southern and south-western China, Vietnam, Laos, Thailand and Myanmar. It is presently comprised of 17 species. In Thailand, only two species have been recorded, namely *G. carinensis* (Boulenger) and *G. seesom* (Massui, Khonsue, Panha & Eto). The latter of these two species is currently known to be endemic to the country.

New information

Based on recent field work conducted in 2019 in Doi Phu Kha National Park, Nan Province of northern Thailand, we are reporting two new records of the genus *Gracixalus*, *G. quangi* and *G. yunnanensis*, from Thailand, based on morphological and molecular evidence. In addition, this is the first study to report on the identification of a female specimen of *G. yunnanensis*. Furthermore, morphological data and natural history notes of the aforementioned species in Thailand have been provided, along with updated locations for the distribution of both species.

Keywords

Gracixalus quangi, *G. yunnanensis*, new record, 16s rRNA, Nan Province

Introduction

The bushfrog genus *Gracixalus* is known from southern and south-western China, Vietnam, Laos, Thailand and Myanmar. Currently, 17 nominal species are known (Nguyen et al. 2020a, Frost 2020). This genus is characterised by a small body size (SVL < 50 mm), the absence of vomerine teeth, the presence of a dark X or an inverted V-shaped figure on the dorsum and the absence of serrated dermal fringes on the limbs (Rowley et al. 2011, Yu et al. 2019, Nguyen et al. 2020a). To date, only two species, namely *G. carinensis* (Boulenger) and *G. seesom* Matsui, Khonsue, Panha & Eto (currently endemic to the country), have been reported in Thailand (Matsui et al. 2015, Frost 2020).

Quang's bushfrog *G. quangi* was recently described by Rowley et al. (2011) from Pu Hoat Nature Reserve, Nghe An Province, Vietnam. The species has since been reported from Son La, Hoa Binh and Thanh Hoa Provinces in Vietnam, Vientiane and Xaisomboun Provinces in Laos. Additionally, it is known to be found in the Yunnan Province of China (Rowley et al. 2011, Pham et al. 2019, Nguyen et al. 2020b, Liu et al. 2020). Currently, this species is listed as vulnerable (VU) in the IUCN Red List of Threatened Species (IUCN SSC Amphibian Specialist Group 2015). To date, specimens of the bushfrog *G. yunnanensis* have been described, based only on adult males that had been observed in south-western and southern Yunnan in China; however, molecular data have revealed that this species is also distributed in Laos (Houaphan Province) and Vietnam (Lao Cai and Nghe An Provinces) (Yu et al. 2019).

During recent fieldwork in Nan Province of northern Thailand, we collected specimens that can morphologically be assigned to the genus *Gracixalus*. The results of our morphological comparisons and molecular analysis indicate that these specimens should be referred to as *G. quangi* and *G. yunnanensis*. Here, we have reported on two bushfrog species, namely *G. quangi* and *G. yunnanensis*, for the first time from Thailand.

Materials and methods

Sampling

Field surveys were conducted in Nan Province in December 2017 (Fig. 1). Live specimens were collected and photographed before being euthanised using a 15% solution of benzocaine prior to fixation and storage in 75% ethanol. The specimens were then fixed or made fast with 10% formalin for 24 h and stored in 75% ethanol. Tissue samples were taken for genetic analysis prior to preservation and were stored in 95% ethanol. Specimens and tissues were subsequently deposited in the herpetological collections of the School of Agriculture and Natural Resources, University of Phayao (AUP), Phayao, Thailand.



Figure 1. [doi](#)

Map showing the distribution of *G. quang* and *G. yunnanensis* and location of the studied population (red circle), Type locality of *G. quang* (yellow star) and *G. yunnanensis* (red star).

Morphological characteristics

Measurements were taken to the nearest 0.1 mm using digital calipers. Abbreviations follow Matsui (1984) SVL: Snout-vent length, HL: Head length, HW: Head width, SL: Snout-length, EL: Eye length, TD: horizontal diameter of tympanum, N-EL: Nostril-eyelid length, IND: Internarial distance, IOD: Interorbital distance, UEW: Upper cycloid width, FLL: Forelimb length, LAL: Lower arm length, HAL: Hand length, 1FL: First finger length, IPTL: Inner palmar tubercle length, OPTL: Outer palmar tubercle length, 3FDD: Third finger disc diameter, HLL: Hind limb length, TL: Tibia length, FL: Foot length, IMTL: Inner metatarsal tubercle length, 1TOEL: first toe length, 4TDD: fourth toe disc diameter, OMTL: Outer metatarsal tubercle length. Other abbreviations include: Mt.: Mountain, NP: National Park, NR: Natural Reserve, asl: above sea level. Sex was determined by the presence of nuptial pads, vocal sac and by gonadal inspection.

Molecular analysis

Total genomic DNA was extracted from liver tissue using a DNA extraction kit provided by Tiangen Biotech (Beijing) Co. Ltd. A 550 bp fragment of the mitochondrial 16S rRNA gene was amplified for each sample using the primer pairs L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCGGTCTGAACTCAGATCACGT-3') (Wang et al. 2018). PCR amplifications were performed in a 20 µl reaction volume with the following cycling conditions: an initial denaturing step at 95°C for 4 min, 33 cycles of denaturing at 94°C for 30 s, an annealing step at 52°C for 30 s, an extending step at 72°C for 1 min and a final extending step of 72°C for 7 min. PCR products were purified with spin columns. The purified products were sequenced with both forward and reverse primers using a BigDye Terminator Cycle Sequencing Kit according to the guidelines provided by the manufacturer on an ABI Prism 3730, employing automated DNA sequences. All sequences have been deposited in GenBank (Table 1).

Table 1.

Samples used in molecular analysis of this study.

| Species | Locality | Cataloguenumber | GenBank No. |
|-----------------------------|-----------------------------------|-----------------|--------------------------|
| <i>Gracixalus ananjevae</i> | Pu Hoat NR, Nghe An, Vietnam | VNMN 03012 | JN862546 |
| <i>G. ananjevae</i> | Wenshan, Yunnan, China | 03320Rao | GQ285669 |
| <i>G. gracilipes</i> | Pingbian, Yunnan, China | KIZ 060821196 | EF564523 |
| <i>G. gracilipes</i> | Ha Giang, Vietnam | AMNH A163897 | DQ283051 |
| <i>G. guangdongensis</i> | Hunan, China | CIB HN201108200 | LC011936 |
| <i>G. guangdongensis</i> | Mt. Nankun, Guangdong, China | SYS a004902 | MG520193 |
| <i>G. jinggangensis</i> | Mt. Jingjang, Juangxi, China | SYS a003186 | KY624587 |
| <i>G. jinxiuensis</i> | Mt. Dayao, Jinxiu, Guangxi, China | KIZ 060821126 | EU215525 |
| <i>G. jinxiuensis</i> | Mt. Dayao, Jinxiu, Guangxi, China | KIZ 060821013 | EF564524 |
| <i>G. lumarius</i> | Kon Tum Province, Vietnam | AMS R176202 | KF918412 |
| <i>G. supercornutus</i> | Gia Lai, Vietnam | AMS R176287 | KT374016 |
| <i>G. nonggangensis</i> | Nonggang NR, Guangxi, China | NHMG20091009 | JX841319 |
| <i>G. nonggangensis</i> | Nonggang NR, Guangxi, China | NHMG1005046 | JX841320 |
| <i>G. nonggangensis</i> | Ha Lang, Cao Bang, Vietnam | IEBR A.2012.2 | JX896681 |
| <i>G. nonggangensis</i> | Ha Lang, Cao Bang, Vietnam | VNMN A.2012.3 | JX896685 |
| <i>G. quang</i> | Pu Hoat NR, Nghe An, Vietnam | AMS R173410 | JN862537 |
| <i>G. quang</i> | Pu Hoat NR, Nghe An, Vietnam | AMS R173411 | JN862538 |
| <i>G. quang</i> | Pu Hoat NR, Nghe An, Vietnam | AMS R173417 | JN862539 |
| <i>G. quang</i> | Pu Hoat NR, Nghe An, Vietnam | AMS R173423 | JN862540 |
| <i>G. quang</i> | Pu Hoat NR, Nghe An, Vietnam | AMS R173426 | JN862541 |
| <i>G. supercornutus</i> | Kon Tum, Vietnam | AMS R173395 | JN862542 |

| Species | Locality | Cataloguenumber | GenBank No. |
|--------------------------------|---|-----------------|--------------------------|
| <i>G. supercornutus</i> | Kon Tum, Vietnam | AMS R173396 | JN862543 |
| <i>G. supercornutus</i> | Kon Tum, Vietnam | AMS R173428 | JN862544 |
| <i>G. supercornutus</i> | Kon Tum, Vietnam | AMS R173395 | JN862545 |
| <i>G. quangi</i> | Doi Phu Kha NP, Nan, Thailand | AUP 00388 | MZ268126 |
| <i>G. quyeti</i> | Phong Nha-Ke Bang NP, Quang Binh, Vietnam | VNUH 160706 | EU871428 |
| <i>G. quyeti</i> | Phong Nha-Ke Bang NP, Quang Binh, Vietnam | ZFMK 82999 | EU871429 |
| <i>G. sapaensis</i> | Lai Chau, Vietnam | MNHN 1999.5961 | AY880503 |
| <i>G. sapaensis</i> | Lao Cai, Vietnam | CIB XM-439 | GQ285670 |
| <i>G. seesom</i> | Thong Pha Phum NP, Kanchanburi, Thailand | KUHE 35084 | LC011932 |
| <i>G. tianlinensis</i> | Tianlin, Guangxi, China | NHMG 1705015 | MH117960 |
| <i>G. tianlinensis</i> | Tianlin, Guangxi, China | NHMG 1705016 | MH117961 |
| <i>G. trieng</i> | Mt. Ngoc Linh Kon Tum, Vietnam | AMS R176206 | MT328246 |
| <i>G. trieng</i> | Mt. Ngoc Linh Kon Tum, Vietnam | UNS 00342 | MT328245 |
| <i>G. yunnanensis</i> | Pu Mat NP, Nghe An, Vietnam | AMS R173454 | JN862547 |
| <i>G. yunnanensis</i> | Jinping, Yunnan, China | KIZ 060821126 | EF564525 |
| <i>G. yunnanensis</i> | Houapan, Laos | KUHE 32453 | LC011937 |
| <i>G. yunnanensis</i> | Doi Phu Kha NP, Nan, Thailand | AUP 01984 | MZ268127 |
| <i>G. yunnanensis</i> | Doi Phu Kha NP, Nan, Thailand | AUP 01985 | MZ268128 |
| <i>G. yunnanensis</i> | Doi Phu Kha NP, Nan, Thailand | AUP 01986 | MZ268129 |
| <i>G. yunnanensis</i> | Doi Phu Kha NP, Nan, Thailand | AUP 01987 | MZ268130 |
| Outgroups | | | |
| <i>Kurixalus effingeri</i> | Okinawa Is, Japan | A120 | DQ468673 |
| <i>Philautus aurifasciatus</i> | Java, Indonesia | ZRC.1.5266 | AY141850 |

Phylogenetic analysis

Sequence alignments were first conducted using Clustal X 2.0 (Wang et al. 2018) with default parameters. Alignment was then checked and manually revised. Data were tested in jmodeltest v.2.1.2 using Akaike and Bayesian Information Criteria to provide the best-fitting nucleotide substitution models as GTR+I+G. Sequence data were analysed using Maximum Likelihood (ML) implemented in RAxML GUI 1.3. For ML analysis, a bootstrap consensus tree was inferred from 1,000 replicates and was used to represent the evolutionary history of the taxa that were analysed. Branches that corresponded to the partitions reproduced in less than 50% of the bootstrap replicates were collapsed. For Bayesian Inference (BI) analysis, two independent runs with four Markov Chain Monte Carlo simulations were performed for ten million iterations and sampled every 1,000th iteration. The initial 25% of the samples were discarded as the burn-in. The convergence of the Markov Chain Monte Carlo simulations was assessed using Tracer v.1.4. We also

calculated the pairwise sequence divergence, based on uncorrected p-distance using MEGA 7.

Taxon treatments

*Gracixalus quang*i Rowley, Dau, Nguyen, Cao & Nguyen, 2011

Material

- a. scientificName: *Gracixalus quang*i; class: Amphibia; order: Anura; family: Rhacophoridae; genus: *Gracixalus*; specificEpithet: *quangi*; scientificNameAuthorship: Rowley, Dau, Nguyen, Cao & Nguyen, 2011; country: Thailand; countryCode: TL; stateProvince: Nan; locality: Doi Phu Kha; verbatimElevation: 1269; verbatimCoordinates: WGS84; verbatimLatitude: 19°11.59 N; verbatimLongitude: 101°04.52 E; eventRemarks: collected by L. Sengvilay, P. Pawangkhanant and C. Suwannapoom; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: AUP-00388; basisOfRecord: preserved specimen

Table 2.
Measurement (in mm) and proportions of the series of *Gracixalus quang*i and *G. yunnanensis* collected from Nan Province, Thailand (see Materials and Methods section for list of abbreviations).

| Characters | <i>G. quang</i> i | <i>G. yunnanensis</i> | | | | | |
|------------|-------------------|-----------------------|-----------|-----------|--------------------|----------------------|-----------|
| | AUP-00388 | AUP-01984 | AUP-01985 | AUP-01987 | Min–Max (n = 3) | Mean ± SD (n = 3) | AUP-01986 |
| Sex | Male | Male | Male | Male | | | Female |
| SVL | 25.9 | 32.3 | 38 | 35.7 | 32.3–38.0 | 35.4 ± 2.9 | 39.3 |
| HL | 10.8 | 12.5 | 13.1 | 13.3 | 12.5–13.3 | 13.0 ± 0.4 | 13.2 |
| SL | 4.2 | 4.3 | 5.8 | 5.5 | 4.3–5.8 | 5.2 ± 0.8 | 5.7 |
| EL | 4.7 | 5.0 | 5.2 | 4.8 | 4.8–5.2 | 5.0 ± 0.2 | 5.1 |
| N-EL | 2.5 | 3.1 | 3.3 | 3.5 | 3.1–3.5 | 3.3 ± 0.2 | 3.6 |
| HW | 9.1 | 11.6 | 13.4 | 13.6 | 11.6–13.6 | 12.8 ± 1.1 | 14.6 |
| IND | 1.9 | 3.4 | 3.4 | 3.7 | 3.4–3.7 | 3.5 ± 0.2 | 4.1 |
| IOD | 2.7 | 3.7 | 4.4 | 4.1 | 3.7–4.4 | 4.1 ± 0.4 | 4.4 |
| UEW | 2.3 | 2.6 | 2.9 | 2.8 | 2.6–2.9 | 2.8 ± 0.2 | 2.7 |
| FLL | 16.1 | 21.8 | 25.1 | 26 | 21.8–26.0 | 24.3 ± 2.3 | 27 |
| LAL | 13.1 | 14.8 | 17.5 | 18.1 | 14.8–18.1 | 16.8 ± 1.8 | 18.7 |
| HAL | 9.5 | 8.2 | 11.2 | 10.6 | 8.2–11.2 | 10.0 ± 1.6 | 11.2 |
| 1FL | 4.3 | 3.8 | 4.7 | 4.8 | 3.8–4.8 | 4.4 ± 0.5 | 5.1 |
| IPTL | 1.3 | 1.5 | 1.7 | 1.7 | 1.5–1.7 | 1.6 ± 0.1 | 1.8 |
| OPTL | 1.9 | 1.8 | 2.2 | 2.0 | 1.8–2.2 | 2.0 ± 0.2 | 2.1 |
| 3FDD | 1.5 | 1.1 | 1.3 | 1.1 | 1.1–1.3 | 1.2 ± 0.1 | 1.4 |
| HLL | 44.3 | 47.8 | 57.2 | 55.9 | 47.8–57.2 | 53.6 ± 5.1 | 41.6 |
| TL | 14.5 | 14.8 | 17.9 | 17.5 | 14.8–17.9 | 16.7 ± 1.7 | 17.8 |

| Characters | <i>G. quangi</i> | <i>G. yunnanensis</i> | | | | | |
|------------|------------------|-----------------------|-----------|-----------|--------------------|----------------------|-----------|
| | AUP-00388 | AUP-01984 | AUP-01985 | AUP-01987 | Min–Max (n = 3) | Mean ± SD (n = 3) | AUP-01986 |
| Sex | Male | Male | Male | Male | | | Female |
| FL | 17.3 | 19.9 | 23.3 | 23.6 | 19.9–23.6 | 22.3 ± 2.0 | 23.9 |
| IMTL | 1.1 | 1.8 | 1.7 | 1.8 | 1.7–1.8 | 1.8 ± 0.0 | 1.9 |
| 1TOEL | 3.8 | 5.2 | 5.4 | 4.6 | 4.6–5.4 | 5.1 ± 0.4 | 5.3 |
| 4TDD | 1.1 | 1.3 | 1.4 | 1.2 | 1.2–1.4 | 1.3 ± 0.1 | 1.4 |
| TD | 0.8 | 2.7 | 2.9 | 2.7 | 2.7–2.9 | 2.8 ± 0.1 | 2.9 |
| OMTL | 2.0 | 2.6 | 2.3 | 2.4 | 2.3–2.6 | 2.4 ± 0.1 | 2.5 |
| HL/SVL | 0.42 | 0.39 | 0.35 | 0.37 | 0.35–0.39 | 0.37 ± 0.02 | 0.34 |
| HW/SVL | 0.35 | 0.36 | 0.35 | 0.38 | 0.35–0.38 | 0.36 ± 0.02 | 0.37 |
| HL/HW | 1.19 | 1.08 | 0.98 | 0.98 | 0.98–1.08 | 1.02 ± 0.00 | 0.91 |
| TL/SVL | 0.56 | 0.46 | 0.47 | 0.49 | 0.46–0.49 | 0.47 ± 0.02 | 0.45 |

Description

The morphological characteristics of specimen (n = 1) obtained from Nan Province agreed with the descriptions published by Rowley et al. (2011), Pham et al. (2019), Nguyen et al. (2020b), Liu et al. (2020), body size small in male (SVL 25.9 mm) and data measurements of specimens presented in Table 2. Head longer than wide, snout pointed, projecting beyond margin of the lower jaw, canthus rostralis distinct, loreal region slightly concave, nostrils closer to tip of snout than eyes, interorbital distance wider than internarial distance and upper eyelid, pupil oval, horizontal, pineal ocellus, absent tympanum slightly distinct, rounded, vomerine teeth absent, tongue notched posteriorly, external subgular vocal sac. Forelimbs moderately robust, tips of fingers enlarged into round discs with circum-marginal grooves, relative length of fingers I < II < IV < III, fingers free of webbing, subarticular tubercles prominent, rounded, formula 1, 1, 2, 2, nuptial pad present on finger I. Hind limbs: tips of toes enlarged into round disc with circummarginal grooves, relative length of toes I < II < III < V < IV, discs of toes slightly smaller than those of fingers, webbing between toes well developed, subarticular tubercles distinct, rounded, formula 1, 1, 2, 3, 2, inner metatarsal tubercle present, outer metatarsal tubercle absent, heels overlapping when legs at right angles to body. Skin: dorsal surface of head, body, thigh and shank with small tubercles, largest and most concentrated on eyelids, supratympanic fold present, throat and chest smooth, ventral surface of thighs and belly coarsely granular, pointed projection at tibiotarsal articulation present.

Colouration in life

Dorsal surface dark brown, body olive green with one dark brown line running through the eye rim to the upper arm, small scattered white and yellow black spots on the sides of the body, translucent pale green colour on the loreal region from the snout to the area under the eye, pale blue under the supratympanic fold with the abdominal surface

appearing as opaque white, surface neck faint green with pale patches, ventral surface of the upper arm and thigh appears pale green and translucent with small dark brown spots, bright yellow on the inner surfaces of the thighs and groin with a patch posterior to the insertion of arms, thighs and shanks appearing as bright yellow, upper arms appear ventrally translucent pale green (Fig. 2).

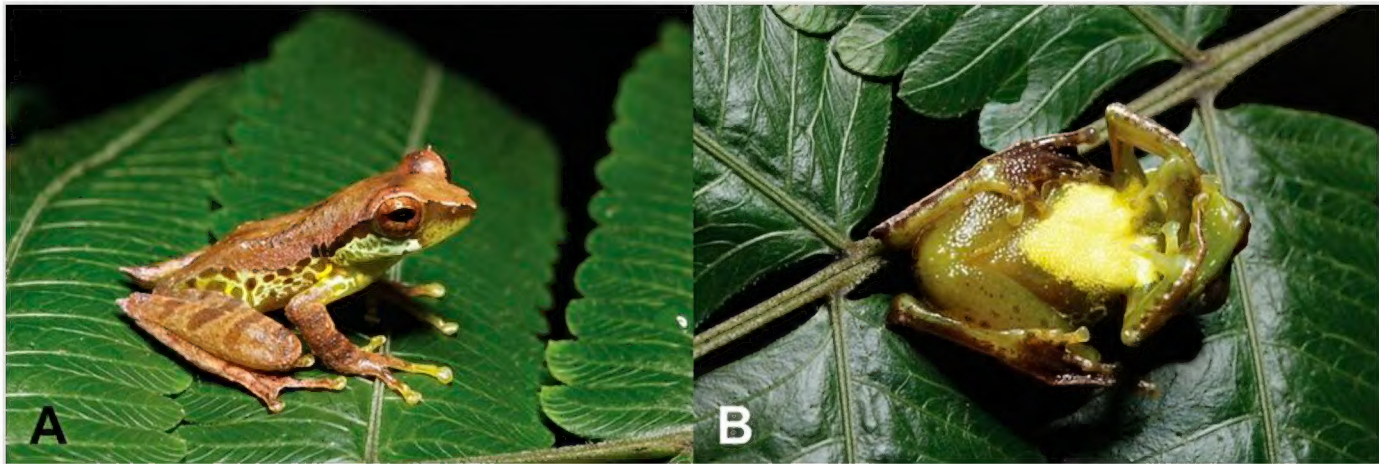


Figure 2. [doi](#)

Male *G. quangi* (AUP-00388) collected from Doi Phu Kha NP, Nan Province, Thailand. **A.** Lateral view; **B.** Ventral view. Photo by P. Pawangkhanant.

Distribution

This species was previously known from the western side of the Red River: northern Vietnam (Pu Hoat NR. in Nghe An Province, Copia NR. in Son La Province, Hang Kia-Pa Co NR. and Ngoc Son-Ngo Luong NR. in Hoa Binh Province, Xuan Lien NR. in Thanh Hoa Province), northern Laos (Kasy District in Vientiane Province and Long Cheng District in Xaisomboun Province) and southern China (Yiwu NR., Mengla Country in Yunnan Province) (Rowley et al. 2011, Pham et al. 2019, Nguyen et al. 2020b, Liu et al. 2020). Notably, this is the first record of this species from Thailand and represents the westernmost distributional limit of this species.



Figure 3. [doi](#)

Habitat of *G. quangi* (**A**) and *G. yunnanensis* (**B**) located in Doi Phu Kha NP., Nan Province, Thailand. Photos by P. Pawangkhanant.

Ecology

A single individual was observed at night at 21:00 h to be sitting on a large leaf of the *Zingiberaceae* ssp. plant that was located about 1.5 m above the ground and close to a rocky stream. The stream was covered with large *Musa acuminata*, a mix of *Dendrocalamus copelandii* and various herbaceous plants. Other amphibian species found in the area included *Leptobrachella minima* (Taylor), *Megophrys* sp., *Sylvirana cubitalis* (Smith) and *Rhacophorus rhodopus* Liu & Hu (Fig. 3A)

Gracixalus yunnanensis Yu, Li, Wang, Rao, Wu & Yang, 2019

Materials

- a. scientificName: *Gracixalus yunnanensis*; class: Amphibia; order: Anura; family: Rhacophoridae; genus: *Gracixalus*; specificEpithet: *yunnanensis*; scientificNameAuthorship: Yu, Li, Wang, Rao, Wu & Yang, 2019; country: Thailand; countryCode: TL; stateProvince: Nan; municipality: Amphoe Pua; locality: Doi Phu Kha NP., near Phu Kha Village; verbatimElevation: 1678; verbatimLatitude: 19°10.40 N; verbatimLongitude: 101°06.44 E; verbatimCoordinateSystem: WGS84; eventDate: 9November 2019; eventRemarks: collected by C. Suwannapoom, P. Pawangkhanant and S. Lorphengsy; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: AUP-01984; basisOfRecord: preserved specimen
- b. scientificName: *Gracixalus yunnanensis*; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: AUP-01985; basisOfRecord: preserved specimen; dynamicProperties: collection date, collector and location as the AUP-01984
- c. scientificName: *Gracixalus yunnanensis*; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: AUP-01986; basisOfRecord: preserved specimen; dynamicProperties: collection date, collector and location as the AUP-01984
- d. scientificName: *Gracixalus yunnanensis*; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: AUP-01987; basisOfRecord: preserved specimen; dynamicProperties: collection date, collector and location as the AUP-01984

Description

Morphological characteristics of specimens (n = 4) collected from Nan Province agreed with the description of Yu et al. (2019), body size small (SVL 32.3–38.0 mm) in males (n = 3), 39.3 mm in female specimens (n = 1), other relevant data measurements of all specimens are presented in Table 2. Head wider than long, snout rounded and projecting slightly beyond the margin of the lower jaw in ventral view, canthus rostralis rounded, loreal region oblique, slightly concave, nostrils oval, protuberant and closer to tip of snout than eye, interorbital distance wider than internarial distance and upper eyelid, pupil oval, horizontal, pineal ocellus absent, tympanum distinct, round, supratympanic fold distinct, vomerine teeth absent, tongue notched posteriorly, external subgular vocal sac. Fore limb relatively robust, tips of all fingers expanded into discs with circummarginal grooves, relative length of fingers I < II < IV < III, webbing between fingers rudimentary, subarticular tubercles prominent, rounded, formula 1, 1, 2, 2 supernumerary tubercles present, inner metacarpal tubercle present, outer metacarpal tubercle present, nuptial pads present on finger I. Hind limbs: relative length of toes I <

II < III < V < IV, tips of toes expanded into discs with circum-marginal grooves, discs of toes smaller than those of fingers, webbing between toes less developed, subarticular tubercles distinct, formula 1, 1, 2, 3, 2 supernumerary tubercles present, inner metatarsal tubercle present, outer metatarsal tubercle absent, heels overlapping when legs at right angle to body. Skin: dorsal surface scattered with many small conical tubercles on head, upper eyelids and dorsum, flanks of body and dorsal surface of limbs smooth, few small conical tubercles on hindlimbs and forearms, throat, chest, belly and ventre of thigh granulated, few small conical tubercles scattered on ventre of thigh, tibia and forearm.

Colouration in life. Dorsal surface brown with a dark brownish area running across and covering the interorbital area, small dark brown spots on upper eyelid and across the back forming an interrupted marking with a Y-shaped mark on the back starting between the eyes and covering most of head, black eyelids appear pale (Fig. 4A-B). Throat and chest mostly yellowish, ventral surface of throat, chest and anterior belly opaque white, sides of head faint brown, diffused dark brown line under canthus rostralis from eyes to nostrils, no obvious tympanic markings, upper arms and thighs brownish clear, four toes and groin appear bright orange (Fig. 4), toes brown and transparent. The skin on the back of the arms and legs is dark brown. Surface of limbs, including hands and feet, appear pinkish brown (diurnally) or pink (nocturnally).

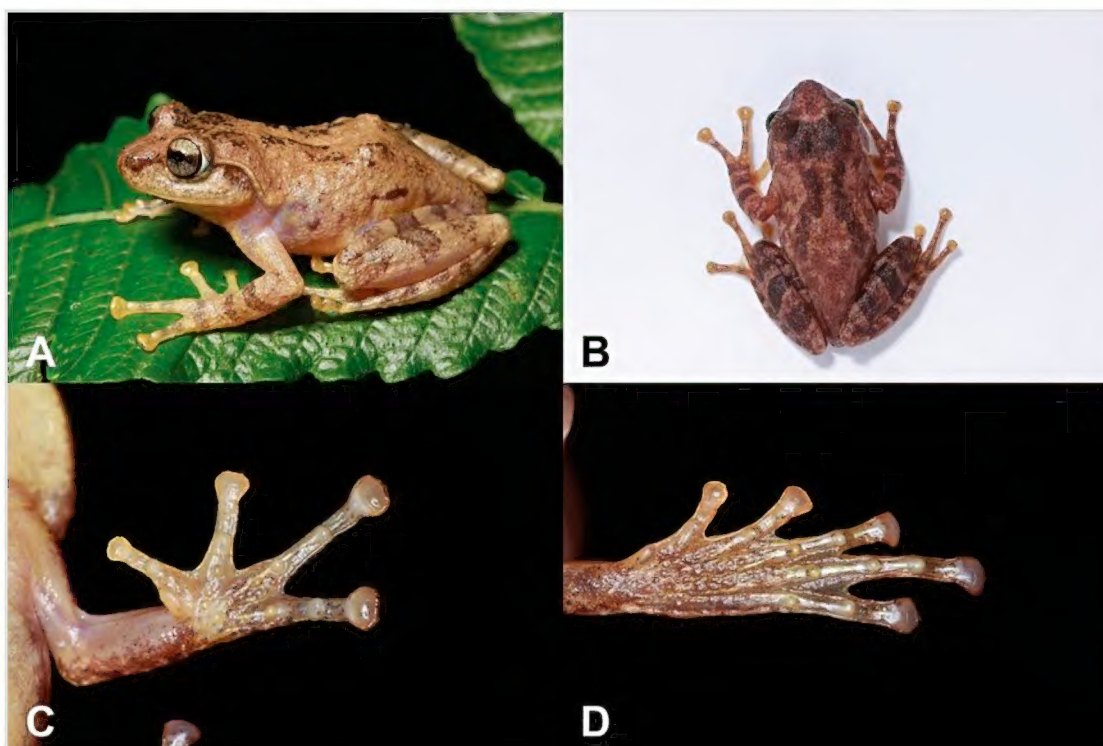


Figure 4. [doi](#)

Male of *G. yunnanensis* (AUP-1985) in life. **A.** Lateral view; **B.** Dorsal view; **C.** Volar view of the left hand; **D.** Plantar view of the right foot. Photo by P. Pawangkhanant.

Remarks. The specimens of *G. yunnanensis* obtained from Nan Province, Thailand differed from those listed in the original description of the specimens collected from Yunnan Province, China (Yu et al. 2019) by having a slightly larger body size in males (32.3–38.0 vs. 26.0–34.2 mm).

Revised diagnosis. SVL 26.0–38.0 mm in male specimens, 39.3 mm in female specimens, distinctive conical asperities on dorsum, snout rounded, no dermal projection, tibiotarsal projection absent, iris bronze, lack of white patch on temporal region, males having an external subgular vocal sac, nuptial pads in finger I and linea masculine, lacking dermal spines on upper eyelids, absent serrated dermal fringes on limbs, tibiotarsal articulation reaching central eye, dorsal surface yellow-brown or red-brown, ventre surface orangish with yellow spots, semi-transparent, finger webbing rudimentary, toe webbing formula: I1.5–2II1.5–2.7III.5–3IV2.5–1.5V (Table 2).

Distribution

This species was previously known from the western part of the Red River located in south-western China (in Xuelin, Fudong, Fazhanhe, Bada and Jinping townships and near Mt. Huanglian in southern Yunnan), northern Laos (Houaphan Province) and northern Vietnam (Lao Cai and Pu Mat NP in Nghe An) (Yu et al. 2019). This is the first record of this species from Thailand and represents the most southwest distributional limits of this species.

Ecology

An individual specimen was observed at night between 22:00 to 24:00 h sitting on the branch of a shrub that was about 1–2 m off the ground in an evergreen forest surrounded by trees near a stream with nearby herbaceous plants. Other amphibian species found in the sympatric area included *Nanorana aenea* (Smith), *Megophrys* sp., *Kurixalus* cf. *verrucosus* (Boulenger), *Theloderma albopunctatum* (Liu & Hu) and *T. gordonii* Taylor (Fig. 3B)

Analysis

Results of the phylogenetic analyses of the subfamily Rhacophorinae were recorded with major nodes being sufficiently resolved (1.0/100, hereafter node support values will be given for BI PP/ML BS, respectively, see Fig. 5). The obtained alignment of the 16S rRNA sequences is ~ 550 bp in length after cutting off both ragged sides. Newly-collected samples of two species, *G. quangi* and *G. yunnanensis*, were gathered from Doi Phu Kha NP, Nan Province, Thailand along with samples collected from Pu Mat NP, Nghe An, Vietnam (AMS R173454), Jinping, Yunnan, China (KIZ 060821126) and samples from Houapan, Laos (KUHE 32453). For the second branch, the specimens of *G. quangi* obtained from Pu Hoat NR, Nghe An, Vietnam (AMS R173410) and Pu Hoat NR, Nghe An, Vietnam (AMS R 173417), were sequenced by the authors of previous studies, namely Rowley et al. (2011), Rowley et al. (2020), Matsui et al. (2015), Yu et al. (2019). Both BI and ML analyses established this lineage as the sister to the clade consisting of *G. quangi* and *G. yunnanensis* with weak support (Fig. 5), whereas the analysis revealed that this lineage is closest to *G. ananjevae* specimens that had been collected from Pu Hoat NR, Nghe An, Vietnam, *G. cf. ananjevae* Wenshan, Yunnan, China and *G. supercornutus*.

Average uncorrected pairwise distances (p-distance) between the new country records and other species ranged from 3.6% (*G. yunnanensis*) to 13.5% (*G. gracilipes*) (Table 3).

Table 3.

The mean pairwise uncorrected p-distance (%) of 16S rRNA gene between species of *Gracixalus*.

| Species | | 1 | 2 | 3 | 4 | 5 | 6 |
|---------|-------------------------|------|------|------|------|-----|---|
| 1 | <i>G. quangi</i> | | | | | | |
| 2 | <i>G. ananjevae</i> | 11.8 | | | | | |
| 3 | <i>G. lumarius</i> | 20.2 | 22.2 | | | | |
| 4 | <i>G. nonggangensis</i> | 13.5 | 11.2 | 16.4 | | | |
| 5 | <i>G. quyeti</i> | 6.62 | 10.7 | 15.5 | 10.7 | | |
| 6 | <i>G. yunnanensis</i> | 9.8 | 7.8 | 13.6 | 9.4 | 8.0 | |

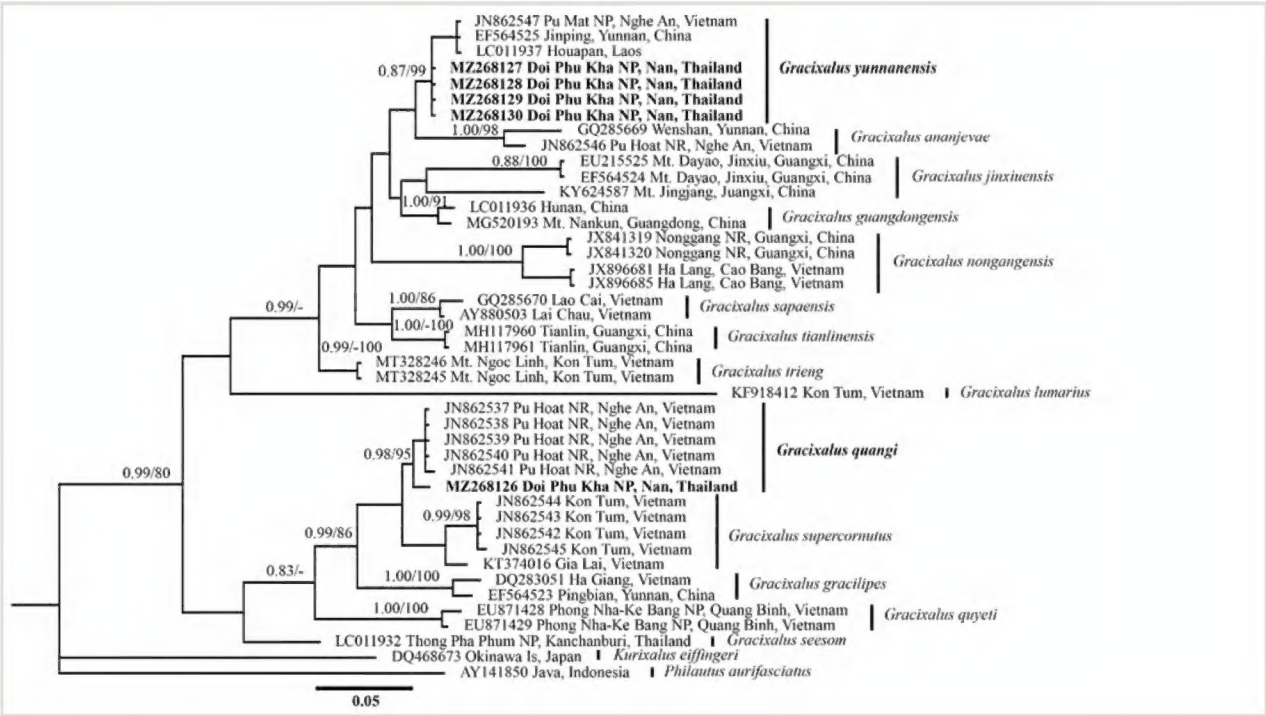


Figure 5. [doi](#)
Maximum Likelihood tree of *G. quangi* and *G. yunnanensis* inferred from (mtDNA) 16S gene.

Discussion

Although there has been a long history of amphibian surveys conducted in Thailand, the diversity of the *Gracixalus* in the country has still been underestimated. Records of two species, namely *G. gracilipes* (Bourret) and *G. carinensis* (Boulenger), were first documented in Doi Inthanon NP, Chiang Mai Province by Nabhitabhata (pers. comm.). Notably, sources of data or the bases for identification are missing. Without examination, specimen consequence would need to be re-evaluated (Nabhitabhata et al. 2000). Similarly, both species were also recorded on the list of amphibians of Thailand by Khonsue and Thirakhupt (2001), Chan-ard (2003), Chan-ard et al. (2011), Chuaynkern and Chuaynkern (2012), but no details were given, other than the general distributional range

of that species. Matsui et al. (2015) described *G. seesom* as being from Kanchanaburi and Changmai Provinces. According to the original description, this species displayed morphological characteristics that are very similar to those of *G. gracilipes*. Thus, they can be easily confused with each other (Matsui et al. 2015). Moreover, the locality type of *G. seesom* is also known to come from Doi Inthanon NP, where *G. gracilipes* has been found (see above). Moreover, intensive surveys were conducted in Doi Inthanon NP and several other forested areas in northern Thailand that have similar habitats on Mae Wong NP in Kamphaeng Phet Province, Huay Kha Khaeng Wildlife Sanctuary in Nakorn Sawan Province and Umphang District in Tak Province. In our surveys, we recorded *G. seesom*, but failed to re-discover *G. gracilipes* (Pawangkhanant NP, published data). Therefore, no strong evidence exists to confirm the presence of *G. gracilipes* in Thailand. Consequently, there is the possibility of the misidentification of *G. seesom*. Thus, we propose to remove it from the list of Thailand's amphibians.

Doi Inthanon, Thailand is recognized as being between the type locality of *G. carinensis* (Karin Hills, Thao and Karin Bia-po, which now belong to Bago Mountain in Bago State, Myanmar; approximately 250 km) and *G. yunnanensis* specimens were recorded at Nan Province in this study (approximately 275 km). Due to the high morphological similarity between *G. carinensis* and *G. yunnanensis*, it is easy for instances of misidentification to occur (see Yu et al. 2019). Therefore, an investigation of the population of *G. carinensis* at Doi Inthanon NP has been requested for the clarification of species distribution.

G. yunnanensis had recently been described in 2019; therefore, the conservation status of this species has not yet been evaluated. The actual extent of distribution, population trends, reproductive behaviour and ecology of this species remain poorly known. Thus, further research is recommended in order to provide information for future conservation decision-making processes.

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